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CYTOLOGICAL STUDIES IN CYANOPHYCEAE

BY

NATHANIEL LYON GARDNER

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CYTOLOGICAL STUDIES IN CYANOPHYCEAE

A THESIS IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY IN THE UNIVERSITY OF CALIFORNIA
PRESENTED IN NINETEEN HUNDRED AND SIX BY

NATHANIEL LYON GARDNER.

BERKELEY
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1906

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I. INTRODUCTION AND TECHNIQUE.

The somewhat aberrant group of plants known as the Cyanophyceae possesses many exceedingly interesting features, whether considered from a systematic, a physiological, or a cytological standpoint. These plants are representatives of a very ancient group which has adapted itself to the most diverse habitats while retaining its original simplicity of structure. They abound in the cold waters of the north, in the hot springs of the Yellowstone, of New Zealand, and of other parts of the world, where the temperature of the water is near the boiling-point; they grow in shaded places and in the open glare of the sun; are encrusted by

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lime on dripping rocks, penetrate into the shells of various mollusks, thrive equally well in fresh or in salt water, even when the latter is concentrated; in fact there is scarcely a habitat in which they may not be found, where any other forms of chlorophyll-bearing plants can exist. They even possess the power to withstand sudden changes in the environment. *Lyngbyas* growing in salt water may be placed in fresh water without affecting their growth, and will live for a long time if placed in distilled water.

The phylogeny of this group has not been well worked out. It is at present set off by itself with doubtful affinities, but the very fact of its diversity of forms suggests several lines of descent. Considered from the standpoint of external vegetative characters, it approaches somewhat the Chlorophyceae at several points. Some unicellular species of Cyanophyceae very closely resemble some of the unicellular Chlorophyceae. The same is true of some of the filamentous forms. And, finally, so closely do some of the membranaceous forms of the two groups approach each other, that one species (*Prasiola Gardneri* Collins) was placed in the Chlorophyceae until my cytological investigations showed that it belongs to the genus *Merismopedia* of the Cyanophyceae.

Interest is aroused in the cytologist by the group because in it he hopes to discover some clue to the origin of the cell-nucleus. A number of investigators of the group have turned their attention in this direction, and a variety of opinions has arisen concerning it. These cytological studies have also revealed the presence of a variety of structures in the protoplast upon which various interpretations have been placed. The questions of principal interest are: *First,* Is there a nucleus or not? *Second,* If there is a nucleus, what are its constituents, and what is its behavior during cell-division? *Third,* What is the structure of the cytoplasm? *Fourth,* Are chromatophores present or not? *Fifth,* What kind of granules are present, and what is their position in the cell, their structure, and their function?

The far-reaching importance of many of these questions, which are still in controversy, and the writer's especial interest in and familiarity with the group has led to the present cytological investigation.

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(*a*) SPECIES STUDIED.

The writer has been very fortunate in being located where such an abundance and variety of material may be found at all seasons of the year, having collected and studied over a hundred species in this vicinity. Many greenhouses, which are everywhere fertile sources for a number of species, are accessible. Many fresh-water springs and ponds, besides the salt marshes and the Pacific Ocean, teem with members of this group.

This abundance of living material at the writer's disposal all the year round has enabled him to make a free selection of the more typical members of each section of the group.

The principal species here studied are the following:—

Chroococcaceae.

Aphanothece sp.
Synechocystis aquatilis Sauvageau.
Synechococcus curtus Setchell MS.
Merismopedia Gardneri (Collins) Setchell.
Merismopedia glauca (Ehrenberg) Nägeli.

Chamaesiphonaceae.

Dermocarpa fucicola Saunders.
Dermocarpa prasina (Reinsch) Bornet.

Heterocysteae.

Anabaena circinalis Rabenh.
Anabaena Flos-aquae Brebisson.
Anabaena Azollae Strasburger.
Anabaena variabilis Kützing.
Aphanizomenon Flos-aquae Ralfs.
Cylindrospermum licheniforme Kützing.
Cylindrospermum majus Kützing.
Calothrix Braunii B. & F.
Calothrix parietina (Nägeli) Thuret.
Calothrix crustacea Thuret.
Hassallia byssoidea Hassall.
Nodularia armorica Thuret.
Nodularia sphaerocarpa B. & F.
Nostoc microscopicum Carmichael.
Nostoc ellipsosporum Rabenhorst.
Nostoc pruniforme Agardh.
Scytonema Javanicum Bornet.

Homocysteae.

Lyngbya Lagerheimii Gomont.
Lyngbya aestuarii Liebman.
Microcoleus chthonoplastes Thuret.
Oscillatoria margaritifera Kützing.

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Oscillatoria brevis Kützing.
Oscillatoria simplicissima Gomont.
Oscillatoria animalis Agardh.
Oscillatoria proboscidea Crouan.
Oscillatoria chalybea Mertens.
Oscillatoria sancta Kützing.
Oscillatoria limosa Agardh.
Oscillatoria tenuis Agardh.
Oscillatoria curviceps Agardh.
Oscillatoria splendida Greville.
Oscillatoria Bonnemaisonii Crouan.
Oscillatoria geminata Meneghini.
Oscillatoria amphibia Agardh.
Oscillatoria chlorina Kützing.
Oscillatoria laetevirens Crouan.
Oscillatoria acuminata Gomont.
Oscillatoria Okeni Agardh.
Phormidium uncinatum Gomont.
Phormidium favosum Gomont.
Phormidium submembranaceum Gomont.
Symploca Muscorum Gomont.
Spirulina subsalsa Œrsted.
Spirulina major Kützing.
Beggiatoa mirabilis Cohn.
Beggiatoa arachnoidea Rabenh.

(*b*) COLLECTING AND PREPARING MATERIAL.

The habitat of these plants is such that it is difficult to avoid getting sand and various kinds of debris along with almost every collection.

If microtome sections of these small plants are to be made, it is exceedingly desirable to have clean material to cut; and to obtain this is quite troublesome, in some cases almost impossible. This is particularly true of heterocysted forms like *Nostoc,* which imprison sand within the gelatinous thallus. The writer was confronted from the first with this difficulty, which necessitated the invention of some methods whereby suitable material might be procured. A few of these methods are given here.

If *Oscillatorias* and other actively crawling forms which contain sand and debris are to be used, the decantation method is found to be simple and practical. The whole mass is put into a tall jar and thoroughly shaken up, and then allowed to settle for a short time, after which the lighter floating material containing

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the plants is poured off. This process is repeated until the coarse, heavy material is removed. Graniteware pans have been found to be most convenient to hold the plants thus segregated, since they are opaque. This material is placed in the shade and allowed to settle to the bottom of the vessel, whereupon it will crawl to the center in a mass. Any fine debris remaining on the surface is disposed of by floating a sheet of paper on the surface and removing paper and debris together. When placed in direct sunlight oxygen is given off, and, collecting into bubbles, is held in the meshes of the trichomes, so that the whole mass of plants with some fine debris is raised to the surface of the water. From the margin of this mass the plants crawl out and form a thin, clean layer on the surface of the water. This thin, floating layer, which forms a fringe around the mass of material, is carefully trimmed away with a pair of sharp scissors, and placed in a vial with water. In order to get the filaments thus trimmed off into a small mass for embedding, they are repeatedly sucked into and forced out of a pipette until thoroughly broken up. On standing for a few hours in the shade, they will be found to have crawled together again into a single mass, and in this condition may be killed and embedded in paraffin for sectioning.

If an abundance of material is at hand, as compared with the amount of soil and debris, one may, instead of bringing it into the direct sunlight, allow it to remain in the shade on the bottom of the vessel until the plants have crawled out of and formed a thick layer on the debris, from which the clean top layers may be removed with fine forceps, if due care is exercised.

Some of the small species are found scattered among fine debris. In order to get them clean, centrifuging may be resorted to. This separates the debris and leaves the plants still scattered through the water, which may now be decanted and collected by filtration. The best filter paper I know of for this purpose is Schleicher & Schüll's No. 575. This is firm, smooth, and strong, and may be subjected to considerable force in a filter-pump. Chamois skin may also be used for this purpose. If desired, the material may now be taken out and killed, then washed by means of the above apparatus, preparatory to embedding. This is a practical way to wash any form, for it is otherwise difficult to

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prevent the loss of much of the material unless it adheres firmly in a mass.

After the material is washed, it may be placed directly in a dialyzer, and by this means slowly dehydrated, thus preventing shrinkage and also the loss of material.

The embedding and cutting of material is at best a slow process, requiring skill and patience to secure sections suitable for careful study. Experience has shown that many things may be learned about the cell by the use of other methods which facilitate the work and enable one to arrive at the same results in a much shorter time. In fact, it is only in exceptional cases that one needs microtome sections. So far I have been able to demonstrate everything which was revealed by the thin sections without resorting to cutting, and consequently use sections now only to supplement and check results obtained without it. Everything may be seen in the small plants, only a few microns thick, if properly stained.

It is also desirable to study the nucleus as a whole, in longitudinal view, and this has been found to be possible and practical in uncut cells.

Most of the forms have a sufficiently gelatinous wall to cause them to adhere to the slide without any assistance, and may be stained, washed, and permanently mounted within a few minutes, provided a study is to be made without previously killing them. If it is desired to kill them previous to staining, this may be done by placing the slide with the adhering material directly in the killing agent, after which it may be washed, stained, and treated as desired. The more they are handled, the more liable are the specimens to be lost, and if one has forms like *Oscillatoria,* which are not very gelatinous, it is well to place on the slide first a little albumen fixative. *Nostocs, Anabaenas,* etc., of the heterocysted group, do not require this, being sufficiently gelatinous to adhere firmly to the slide.

It is highly desirable to get the material spread out very thin on the slide, and a good method for doing this with the rapidly crawling forms is to allow them to spread out on the surface of the water, as mentioned above, trim off small pieces, which should be carefully floated upon a slide (this may be previously smeared

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with albumin fixative if desired), after which the superflous water is wiped off, and the remainder of the water allowed to evaporate almost to dryness, when the plants are ready for further treatment; or one may place the material, free from debris, in a large drop of water on the desired part of the slide, and allow the water to evaporate. In this condition the plants usually crawl out radially in a thin layer on the slide.

If gelatinous forms are being used, a very small quantity of clean material is placed on the slide and pressed gently with a cover glass into a thin layer, after which the cover should be slid off, not raised straight up.

After staining, the material need not (except in very few cases, *e.g.*, some of the large thin-walled *Oscillatorias*) be run up through the grades of alcohol, for shrinkage will not occur to any detrimental degree if 90 per cent., and then absolute alcohol be dropped on directly with a wash-bottle. For preliminary examination clove oil is best, for this clears the plants and takes out the water, and, if permanent mounts are desired, they can be transferred directly to balsam. Clove oil cannot always be used because some of the stains (*e.g.*, most of the violets) are so readily soluble in it. I have found, however, that in general the violets are not very useful, and, having found better stains, have almost abandoned their use.

End views of cells of the large species of *Oscillatorias* and some other genera are very desirable for getting at the structure of the nucleus. For this purpose a method has been worked out which is vastly superior to the embedding and sectioning method. This method consists in using a killing agent which will affect the cross-cell walls in such a way as to cause the cells to separate easily. In selecting such an agent it was necessary that one be found which would not cause the trichomes to shrink, and also one which could be followed by a variety of good differential stains.

Alcohol will serve this purpose in some of the thin-walled short-celled forms, but the cells do not separate so readily as is desirable, and a large number of them become broken and distorted. The material is killed in 95 per cent. alcohol for half an hour, after which the slides with the mounted plants are placed

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directly in water for a few minutes. On removing the slide and wiping off the superfluous water, a cover glass is placed over the material, and with slight pressure and with a rolling motion, the plants are broken apart and the cells fall down on their flat surfaces. The cover glass should be removed by sliding it to one side, and not by raising it straight up. This helps to spread the cells out in a thin layer, and assists in getting them to adhere to the slide. A little albumin fixative may first be placed on the slide.

Picric acid will bring about the same results as alcohol, and is a good killing agent, but has many disadvantages. Too much time is required to kill the plants, and then much more time is required to get rid of the acid. This is done by washing in alcohol, and care is necessary to prevent the shrinkage of material. Picric acid also has a tendency to loosen the plants from the slide, which then become lost in washing, and, further, it has the disadvantage of not being a killing agent that can be followed by the best differential nuclear stains.

The very best preparation I have thus far found for causing the cells to separate is a strong solution of iodine in strong aqueous solution of potassium iodide. This is an excellent killing agent, acting instantaneously, without shrinking the protoplast to any perceptible degree. Material is mounted on the slide as above described, and a drop of the solution placed upon it, or the whole slide placed in the solution for 10 to 30 minutes, though much longer time will not injure the plants. The material is now washed with 95 per cent. alcohol for about the same length of time, then placed in water for a few minutes, after which it is ready to be treated as above described. The cells break apart readily, adhere to the slide well, and on drying are left round and plump and ready for staining. All of the best differential stains which I have employed for both nucleus and granules may be used after this killing agent with excellent results.

This method is in many ways superior to the microtome sections. There is far less chance for misinterpretation of the shape and location of various cell-organs. One obtains by this method single cells free from other or parts of other cells, a thing which is practically impossible to obtain with microtome sections. Perplexity and confusion arise when one attempts to interpret parts of several or even of two cells in an oblique section.

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The cells of the Principes and Margaritiferae groups of *Oscillatoria* are sufficiently short to allow one to see everything perfectly in properly stained isolated cells.

If one wishes paraffin sections of plants belonging to other genera, it is expedient to use potassium iodide-iodine as a killing agent, for washing and dehydration can be accomplished at the same time. Plants killed in this way can usually withstand being dehydrated rapidly without shrinking.

(*c*) KILLING AND FIXING AGENTS.

Besides the killing agents just mentioned, *viz.*, alcohol, picric acid, and potassium iodide-iodine, the following were tried:—

Flemming's weak and strong chrom-osmic-acetic solutions, saturated aqueous solution of corrosive sublimate, 1 per cent. chromic acid, Hermann's mixture, and iridium chloride.

Potassium iodide-iodine seems to be perfectly satisfactory, hence experimentation with many other solutions has not been resorted to. Flemming's solutions are almost as good as the iodine in the majority of cases, but do not have the advantage of being so easily washed out, and do not dissolve the cross-walls.

(*d*) STAINS AND STAINING.

At the beginning of this investigation two points of technique were specially considered. First, the best killing agents that would produce practically uniform results when followed by a variety of stains; and second, the best differential stains that would uniformly reveal homologous structures in a large enough series of plants to form a basis for the interpretation of the cell structure.

Throughout the work, a Zeiss instrument, with Abbé condenser, 2 mm. apochromatic objective, and 8, 12, and 18 compensating oculars has been used. A white light has been secured by means of a special lamp, and, after being condensed by a large globe of distilled water, has been modified at will by variously colored glass disks.

The following stains have been employed. They are all Grübler's except those marked otherwise.

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LIST OF STAINS.

Red and Orange Stains.

Bordeaux red.
congo red.
ruthenium red.
neutral red.
rubin G., Bausch & Lomb.
rubin S.
erythrosin.
eosin.
carmine, aqueous.
Schneider's aceto-carmine.
Hoyer's picro-carmine.
Woodward's carmine.
Heidenhain's iron haematoxylin.
Ehrlich's haematoxylin.
Böhmer's haematoxylin.
Delafield's haematoxylin.
Mayer's acid haemalum.
fuchsin, aqueous, and alcoholic.
Ziel's carbol fuchsin.
safranin.
sudan III.
orseille G., Bausch & Lomb.
peruvin.
orange G.

Green Stains.

iodine green.
solid green.
light green.
aniline green.
coerulein S.

Blue Stains.

toluidin blue, Chas. C. Riedy, S. F.
china blue.
wasserblau.
victoriablau.
methyl blue.
methylene blue, aqueous.
Loeffler's methylene blue.
blue de Lyon.
thionin, Queen & Co., Phila.
cyanin.
aniline blue.
blue lumière, Chas. C. Riedy, S. F.
alizarinblau.

Brown and Black Stains.

Bismarck brown.
schwarzbraun.
wollschwarz, Chas. C. Riedy, S. F.
nuclear black.

Violet Stains.

crystal violet.
methyl violet.
Hofmann's violet.
säure violet.
gentian violet.
methyl violet 6 B.
methyl violet 5 B.
dahlia.

The method of attacking the problem was first to select from the abundance of material a species with well defined cell-organs to use as a basis of comparison, and also to serve for testing the action of various killing agents and stains. The nucleus was selected as the first object for demonstration, and for this purpose study was begun on the living plant. After careful investigation to ascertain what could be seen by the aid of the highest magnification of the microscope, stains were run under the cover glass

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and their action on the living material noted. Loeffler's methylene blue was used first, and it was soon discovered that while not all the Cyanophyceae cells are alike in cytological characters, one feature of their reaction to stains seems to be common to all, *viz.*, that the central part of the cell uniformly has a stronger affinity for stain than other parts. The central part in many species will be intensely colored even before the cytoplasm through which the stain has to pass is colored. Most specific chromatin stains act in the same way. This leads to the belief that chromatin may be present in this part of the cell. But by this method alone differentiation is not sufficient to enable one to determine the form in which it exists, for very soon other cell-organs become colored, and the whole protoplast becomes diffusely stained. The method afterward employed was to "overstain," and then to remove the superfluous stain and dissolve out that which seems to be held mechanically or in loose combination, observing which parts of the cell give it up first. It was found by this method that the central part of the cell is uniformly the most tenacious of chromatin stains. It was further found that the central part is not homogeneous. The whole mass does not equally become fainter as the stain disappears, but certain parts resist the action of the solvent a long time. In most species thus stained it was found that the granules were the last to give up the stain. Much variation exists among the species as to the degree of differentiation of the central part, and it was not a simple matter to determine at first what is typical. Pursuing the method of staining *intra vitam*, I finally came across *Symploca Muscorum*, which, although having quite a thick sheath, has a very strong affinity for aqueous methylene blue; and in this, as in other species, the central part takes the stain first. Furthermore, the action is almost instantaneous. Staining on the slide with aqueous methylene blue, dehydrating, and mounting in balsam may be accomplished within 5 minutes, with a most excellent differentiation of the central part of the cell. The granules stain red and are scattered within and around the mass of branched thread-like structures which stain blue, while the surrounding cytoplasm remains bluish or greenish, according to the degree of washing out of the stain. In this species was found something

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sharply defined upon which to work, and it was selected as a representative form for a complete and careful study, after which it was used as a basis for comparison with similar structures in other plants. First a number of standard aqueous nuclear stains were used upon the living material. The plants were washed in alcohol and examined in clove oil (just as when stained with methylene blue), with the result that the same structures uniformly appeared. Among the stains used were ruthenium red, neutral red, rubin G., safranin, toluidin blue, china blue, and thionin; and of these the best in general were found to be the blue stains, since they act like double stains, coloring the granules red, thus differentiating them from the other structures, all of which stain blue or bluish. The red stains do not so clearly separate the granules from the thread-like structure in the center of the cell.

To supplement and check this work, plants were killed with several different killing agents, *e.g.,* alcohol, Flemming's solutions, 1 per cent. chromic acid, and potassium iodide-iodine, after which the stains mentioned were applied. Without exception the same structures were revealed as before, but not equally well defined. Chromic acid and Flemming's solution followed by the blue stains do not yield so clear and definite results as these stains do on the living material, nor are the blue stains so good after these killing agents as the red stains. Material killed with potassium iodide-iodine or with alcohol stains about the same as the living material.

Corroborative evidence coming from such a variety of sources as we have here warrants the conclusion that the structures revealed by the methylene blue on the living material are real and not artifact. Furthermore, the selective action of these chromatin stains as indicated here is evidence that the thread-like structure is composed of chromatin.

If in all of the species of Cyanophyceae the chromatin were as easily differentiated as it is in *Symploca Muscorum*, little trouble would be involved in the interpretation of its behavior during cell division, but this is found not to be the case. The abundant granules in the center of the cells of some species, as well as granules embedded in the peripheral cytoplasm, have been a

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source of much confusion, and it was not until I was able to differentiate the granules and stain each kind separately without staining the chromatin, and further to stain the chromatin without staining the granules, that this source of error was removed.

The stains I found to be particularly troublesome in failing to differentiate granules from chromatin and in yielding uniform results on different species are Heidenhain's iron haematoxylin and Flemming's triple stain. Early in the work Ehrlich's haematoxylin was tried without good results, but the poor results were found later to be due to the condition of the stain, which was probably either "unripe" or "over ripe." Later some new Ehrlich's haematoxylin was made up, in which Grübler's haematin was used instead of haematoxylin. The mixture was placed in the sunlight and occasionally shaken for a few days, until the haematin and alum were mostly dissolved.

This stain was found to be the very best differential chromatin stain of the entire series used. It has the advantage of being an excellent killing agent, so that the living material mounted on the slide may be killed and stained at the same time, and has the further advantage of differentiating well after a variety of killing agents. With this it is possible to stain the chromatin without staining any of the granules. To do this requires but a few minutes. If left in the stain longer, the central granules also absorb it and become even more deeply stained than the chromatin. Over-staining may be washed out with acidulated alcohol, 1 per cent. to 2 per cent. HCl in 95 per cent. alcohol.

II. RECENT LITERATURE.

That the structure of the Cyanophyceae cell has been exceedingly difficult to work out and to understand is evidenced by the fact that from the time the task was begun until the present there has been continuous disagreement concerning the nature of the various parts of the protoplast.

It is not the purpose of the writer to go extensively into the literature on the subject in this brief paper. Several good reviews have been published from time to time, among which are

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those by Fischer, Kohl, Macallum, Phillips, and finally Olive's ingenious tabulation of opinions. It is thought expedient, however, to say a few words concerning the nature of the problems, and particularly concerning the recent efforts toward their solution.

Since the nucleus in the higher organisms is looked upon as the governing center of cell activity, the demonstration of the presence or absence of such an organ in the Cyanophyceae cell was naturally one of the first things to claim the attention of the cytologist. The first paper to appear on the subject was by Schmitz in 1879. He decided that the Cyanophyceae cell has a nucleus, but the next year after further investigation he changed his mind. Of the thirty or more writers on the subject, about one-half share Schmitz's final opinion.

The question of interest and importance which naturally follows the demonstration of a cell nucleus is the interpretation of its behavior during cell division. Of the workers who find a nucleus in the Cyanophyceae about one-half claim that its division is mitotic, and the other half that it is amitotic. Thus we have a series of views on this subject ranging from that of Schmitz, who claims that there is no nucleus, to Lawson, who sees a primitive nucleus, and Wager, who sees a nucleus with amitotic division, and finally to the other extreme held by Kohl and several others, who find a nucleus with mitotic division. No less confusion prevails among workers respecting other cell-organs.

Three papers have recently appeared describing mitosis in the Cyanophyceae, but their descriptions signally fail to agree, even when relating to the same species. They are by Kohl, Phillips, and Olive.

Kohl worked upon *Tolypothrix lanata* Wartmann, *Nostoc caeruleum* Lyngbye, *Anabaena catenula* B. & F., *Oscillatoria limosa* Ag., and *Oscillatoria splendida* Greville. His preparations revealed to him the presence of a nucleus without a nuclear membrane, and further that the nucleus divides mitotically. He divides this mitotic process into six stages, *viz.*, first, the formation of a spireme; second, the breaking of this spireme into a definite number of chromosomes, which arrange themselves parallel to the long axis of the cell; third, the constriction of the mitotic figure

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in the middle into the shape of an hour-glass; fourth, the "diaster" stage, in which the chromosomes are divided in the middle into two groups which move apart, and in favorable cases, spindle fibers are seen in the center of the figure; fifth, the daughter chromosomes arrange themselves parallel to each other and to the long axis of the cell; and sixth, the union of the daughter chromosomes into a daughter spireme.

To place before the reader the views of Phillips, I quote from his summary of results:—

1. "The central body of the Cyanophyceae is composed of chromatin and is a true cell nucleus."

2. "This nucleus divides by one of two methods, both of which start upon the karyokinetic history, one going no farther than the net-spireme stage, where it constricts itself into halves, while the other continues farther and forms a rudimentary spindle with rudimentary chromosomes upon linin threads.

4. "The chromatin is arranged on the spireme thread in granules which multiply in number by transverse divisions.

5. "There is no longitudinal splitting of the chromosomes or of the spireme, and in the division of the cell by the method first mentioned above, the two portions of the nucleus are not necessarily equal.

6. "The chromatin is aggregated in hollow vesicles in the resting cell. These vesicles give out their chromatin to the net-spireme very much like the nucleoli of the higher plants, and they may represent it. They are embedded in a granular ground substance.

7. "The cyanophycin granules and slime balls are probably food products. They are located in the chromatophore."

Phillips worked upon "*Nostoc* (three species), *Nostoc* in *Collema, Gloeocapsa polydermatica, Oscillatoria imperator, O. Froelichii, O. nigra, Cylindrospermum macrospermum, Spermosira litorea, Anabaena flos-aquae, Tolypothrix lanata, Rivularia pisum, Gloeotrichia,* and *Spirulina.*"

According to Olive (who has worked upon *Phormidium, Oscillatoria, Nostoc, Cylindrospermum, Calothrix,* and *Gloeocapsa*), there is a nucleus which divides mitotically. He finds "a more or less dense, fibrous, achromatic portion, and, enclosed by this,

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a number of minute, globular or somewhat irregularly shaped chromatin granules.'' He states that both these substances stain with the ''standard nuclear stains, *e.g.*, iron haematoxylin and Flemming's triple stain.'' During mitosis all the characteristic structures are present, including spindle fibers. The chromosomes, which are constant in number for each species, divide crosswise in mitosis.

To explain his interpretation of the spindle and its origin, I quote his sixth conclusion, p. 36: ''The kinoplasmic achromatic portion of the central body constitutes a spindle, which has the shape of a flattened disc in the narrow celled species; and in the longer celled forms, of a broad-poled, somewhat cylindrical figure; or in still others, narrow-poled and spindle-formed. The achromatin consists of a central spindle, which is often very densely fibrous, between the dividing chromosomes, and a portion leading from the chromosomes to the cross-walls, which corresponds to the mantle fibers in position and apparently in function.''

Conclusion seven reads as follows: ''A spireme arrangement of the chromatin granules is also evident in the preliminary nuclear changes. The segmented spireme in *Gloeocapsa* appears to consist of a simple, more or less spiral thread, having about eight chromatin granules held by the linin, and situated in the middle of the cell, with its long axis corresponding to the long axis of the cell.''

''In the filamentous species the spireme apparently consists of a much convoluted thread, and it is further probable that it also is made up of a definite number of distinct chromatin granules, arranged along a linin thread.''

From conclusion nine I quote the following concerning the number of chromosomes:—

''The number of chromosomes in the cells of the same species is constant. . . . Each chromosome apparently corresponds to a simple chromatin granule of the spireme thread. Should this prove to be true, then this presents the hitherto unrecorded phenomenon of a chromosome which consists of a single chromomere.''

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Relative to the splitting of the chromosomes, his eighth conclusion reads: "Finally, the most necessary requirement of mitosis is fulfilled in that a longitudinal fission of the chromosomes occurs. This is plainly evident in the case of *Gloeocapsa,* in which the simple spireme threads divide lengthwise, beginning at the two ends and splitting thence progressively to the middle of the thread. It is highly probable, further, that the splitting of the convoluted spireme of the filamentous species takes place in a somewhat similar manner, since the fission plane begins at the edge of the disc-shaped figure and travels progressively inward to the middle."

Finally I wish to quote his eleventh conclusion concerning the continuity of mitotic activity.

"Although the central body in vegetative filaments seems to be in a continuous state of mitotic activity, it appears occasionally to make a beginning toward a resting condition and to form a delicate membrane and karyolymph. It is probable, however, that the nuclei in the active filaments do not ordinarily approach nearer to a state of rest than the spireme condition or a stage immediately prior to it."

From the foregoing it will be seen that these three authors all agree that there is a nucleus in the Cyanophyceae. They have all worked upon at least one species in common, *Oscillatoria limosa* Agardh (*O. Froelichii* Kützing), yet they have three different interpretations as to the manner in which this nucleus divides. Of these three, Olive seems to have found the greatest conformity in nearly all particulars to the various steps in the typical mitosis of a higher plant cell.

After very careful and prolonged investigation I am unable to subscribe to any of the conclusions just quoted concerning the mitosis of the nucleus in any of the Cyanophyceae upon which they have worked. I have carefully investigated *Oscillatoria limosa* and *O. splendida,* two species upon which Kohl worked, and can speak positively in regard to mitosis in these. There is in no portion of the life-history of the cell any configuration of the chromatin present that can be interpreted as belonging to a mitotic process. The process in the nuclei of these two plants is purely and simply amitotic. Kohl's conclusions are based pri-

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marily upon the nucleus of *Tolypothrix lanata,* but he says on p. 177: "Der Verlauf der mitotischen Kernteilung ist nun genau derselbe bei den beiden *Oscillatoria*-Arten wie bei *Tolypothrix.*"

Zacharias, who has done much work on the cytology of the Cyanophyceae, states that through the kindness of Dr. Kohl he has been permitted to examine these preparations, and after doing so, fails to agree with his conclusions. He remarks concerning these preparations that "Die stärker gefärbten, mannigfach gestalteten Teile der Zentralkörper, wie sie die Präparate und die Figuren der Autoren zeigen, halte ich nicht für chromosomen, sondern für (teilweise auch durch das Präparations-verfahren deformierte) Vorsprünge, Leisten, etc., der Zentralkörper."

Judging from preparations treated in like manner, I agree with Zacharias that these projections, etc., are not chromosomes as ordinarily understood.

Phillips has a more complicated scheme for mitosis than Kohl. In fact, he has two methods, both of which he says may occur in the same species. It would be exceedingly interesting to learn under what condition these two processes take place, and why one should occur at one time, and another at another time.

In either case, the resting condition of the cell is described as having the chromatin distributed within the nucleus in the form of hollow vesicles, and, when nuclear division begins, the first step is the transformation of these chromatin vesicles into a diffuse mass throughout the nucleus. This diffuse mass now collects into a network of threads along which, particularly at the junction of the threads, are arranged chromatin granules, which increase in number by division in a plane perpendicular to the thread. At this stage one of two things may happen. The network is either constricted in the middle into two parts after being drawn to the "equator of the cell," or it is resolved into a spireme thread. If the former case prevails, the two masses are drawn into the daughter cells and separated from each other by the ingrowing cross-wall. Of their subsequent history nothing is said.

If the other process prevails, after the orientation of the spireme in the direction of the long axis of the cell, it breaks up into a number of "segments" or "chromosomes." At this juncture

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the chromatin arrives at a place of variability. There are either several fine chromosomes, or the segments may consolidate into a few large chromosomes. The next step is the simultaneous cross-division of these parallel chromosomes in the middle. The cell-wall grows across and completes the division by cutting in two the "linin connectives," and forming the two daughter cells. The fate of the daughter chromosomes is here again uncertain. They are either resolved into a new network or changed back again to the chromatin vesicles.

It is to be noted in this scheme of mitosis that extreme variability prevails.

The karyokinetic process as described by Olive is here briefly outlined.

Chromatin granules are embedded in the achromatic substance of the never resting cell. These granules arrange themselves along a linin thread, which becomes the "segmented spireme" in *Gloeocapsa* or the "convoluted spireme" in the filamentous forms. The granules of which the spireme thread is composed constitute the chromosomes of which there is a definite number in the cell of each species. The next step after the formation of the spireme is its longitudinal splitting, thus accomplishing the equal division of all the hereditary qualities. The mantle fibers extending to the cross-walls attach themselves to each dividing daughter granule or chromosome, and the whole mass is pulled into the daughter cell, after which the remainder of cell division is accomplished by the ingrowing of the cell-wall, and separation of central spindle and cytoplasm. The daughter granules, or chromosomes, are still embedded within the achromatic substance, and a second division proceeds exactly as before. This second division may begin before the first one is completed, and even a third and a fourth division may be in progress before the original one is accomplished.

From these brief summaries it will be noticed that there are widely different views, not only as to the number of different steps involved in the mitotic process, but also as regards the essential manner in which these processes are carried out. A clearer comparative understanding of the three processes of mitosis just briefly outlined may be obtained from the following treatment of

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the subject in which the views on each structure and process are brought together.

All three workers agree in calling the "central body" a nucleus, and Kohl and Phillips agree that it may enter upon a state of rest, though Kohl thinks this is very rare, while Olive finds it in continuous mitotic activity, except in spores and heterocysts.

Structure of the Nucleus.

Kohl. The nucleus is composed of a ground substance, "Centralkörner," and chromatin.

Phillips. The "central body," the nucleus, is composed of chromatin (p. 325, first conclusion) and a "finely granular ground substance" (p. 284).

Olive. The "central body," the nucleus, "consists of a more or less dense, fibrous achromatic portion," and "a number of minute globular, or somewhat irregularly shaped chromatin granules" (p. 35), and sometimes central granules.

The Ground Substance.

Kohl. The ground substance sends out radiating threads to the cell-wall.

Phillips. The granular ground substance permeates the nucleus and sends out finely granular kinoplasmic-like processes through the "chromatophore," which pass out to the cell-wall and form the central part of the "protoplasmic ciliary-like growths" (p. 284), and actually pierce the cell-wall, and "cause the movements of the trichomes" (p. 326).

Olive. The shape of the ground mass varies with the shape of the nucleus.

Chromatin.

All three agree that chromatin is present in the nucleus of the Cyanophyceae, but of the form it assumes and of its behavior the following is said:—

Kohl. "Wie jeder Zellkern, so enthält auch der Zentralkörper der *Cyanophyceen* Substanz, welche sich gewissen Färbungsmitteln gegenüber anders verhält, als die Grundmasse des Zentralkörpers. Da diese sich distinkt färbende Substanz sich

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ausnahmslos in den Gebilden wieder findet, die wir Kernfaden und Chromosomen nennen, so darf ich diese Substanz wohl als Chromatin bezeichnen'' (p. 122).

Phillips. According to this author, the chromatin exists in the ''central body,'' the nucleus, of the resting cell, in the form of ''hollow vesicles'' (p. 284). These hollow vesicles he identifies as being the same as the ''slime balls'' of other authors, and the ''red granules'' of Bütchli (p. 282).

Olive. Globular, or irregularly shaped, presumably solid granules (p. 35).

The Behavior of Chromatin at the Beginning of Cell Division.

Kohl. ''Der dicke Kernfaden durchzieht in Windungen den Kern'' (p. 173).

Phillips. The chromatin of the ''vesicles'' becomes diffused through the ''central body,'' the nucleus, and then forms itself ''into a more or less loose network'' (p. 295); and ''the chromatin is arranged on the spireme thread in granules'' (p. 325, fourth conclusion).

Olive. The chromatin granules become embedded in a ''segmented spireme'' in *Gloeocapsa*, and in a ''convoluted spireme'' in other species.

Spireme.

Kohl. The spireme is formed by the union of chromosomes from the preceding cell division, and is divided crosswise into a *definite* number of pieces, preparatory to a new cell division (p. 173).

Phillips. Two processes are possible with the ''loose network.'' It is either directly constricted into two portions (p. 296), or it may form into ''a single coiled linin thread or spireme,'' which breaks up into an *indefinite* number of pieces which arrange themselves parallel to each other and to the long axis of the cell (p. 296). Of the further history of the thread in the first process we have no record.

Olive. The spireme never breaks into chromosomes or segments. Each chromatin granule in the spireme is looked upon as constituting a ''chromosome'' which never loses its identity as such throughout the life history of the plant (p. 36).

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Chromosomes.

Kohl. This author has a *definite* number of chromosomes in each species which, judging from his illustrations in tables *i* and *k*, separate simultaneously. The division is crosswise and in the middle (p. 173).

Phillips. An *indefinite* number of chromosomes is present, in some cases many fine ones, and in other cases about two coarse ones. In either case they divide crosswise in the middle simultaneously (p. 297).

Olive. A *definite* number of chromosomes is present in each species. These are divided in the same plane as the spireme in which they are enclosed or held fast, the division beginning at the ends of the "segmented spireme" in *Gloeocapsa*, and progressing toward the center, and beginning around the margin of the disc in the "convoluted spireme" of the short-celled form, progressing toward the center of the disc dividing the granules hit or miss as they are reached (p. 36).

Spindle.

Kohl. "Innerhalb der eingeschnürten Partie des Kernes konnte ich bei geeigneter Fixierung und Färbung deutlich Spindelfasern in wechselnder Zahl erkennen" (p. 173).

Phillips. An "open" spindle is formed by the segments of the spireme (pp. 296, 297).

Olive. The kinoplasmic achromatic portion of the central body constitutes a "spindle," the shape of which varies with the shape of the cell. It consists of two parts, one extending between the dividing chromosomes and the other attached to the chromosomes and extending to the cross-walls of the cell (p. 36).

Nuclear Membrane.

Kohl and Phillips find no nuclear membrane, but Olive finds a delicate one in spores.

Granules.

Two kinds of granules have been observed by each of these writers, *viz.*, the "slime globules" and "cyanophycin granules."

From the doubtful manner in which some of these conclusions

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are expressed, and the appearance of the illustrations representing the material from which the conclusions were drawn, it seems that the existence of a karyokinetic process as here set forth is exceedingly doubtful.

From my experience in working on the same organisms it is manifest that the chief trouble which has confronted these investigators and obscured the truth, thus preventing a unanimous verdict, is the failure to discover a method which would clearly, definitely, and unmistakably differentiate the structures which are present. Because of this state of affairs, they have attempted to harness to a comparatively simple process of nuclear division the complicated mitotic process of the higher organisms, with its formation of spireme, chromosome, spindle, etc.

III. THE CELL CONTENTS.

The study of the protoplast of the Cyanophyceae cell has revealed to the writer the presence of the following structures, some of which are constant, while others may or may not be present, according to conditions which have not yet been definitely determined. First, there is a structure in the center of the cell which occupies a larger or smaller portion of the cell, according to the species; and its form is largely determined by the form of the cell. The degree of its demarcation from the surrounding elements is a variable factor. In *Oscillatoria margaritifera,* for example, the line of separation is not sharply defined, while in *Cylindrospermum* the line is as sharply defined as in some of the higher plants. On account of its appearance in the living cell, its reaction to stains and its chemical composition, I have decided to call this the cell-nucleus. Second, surrounding the nucleus and extending to the cell-wall is the cytoplasm, which varies in structure slightly in different species, as well as in the same species, according to the amount of the third kind of structure present, *viz.,* the granules. Incidentally, other structures have been noted in certain species, for example, more or less cylindrical refractive bodies in the cytoplasm of *Oscillatoria chalybea,* to which I have given no name nor ascribed any function. These do not occur in every collection of the species, but when present in a collection

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they are to be found in every plant. Since the other characters are constant in all collections, these bodies probably do not furnish a specific character, but only a physiological one. These are probably the refractive bodies spoken of by Gomont in his description of the species. Another constituent noted is the refractive substance connecting the heterocysts to vegetable cells, called "Verschlusskörper" by Kohl.

We will now take up the consideration of these various structures more in detail.

(*a*) THE NUCLEUS.

It has previously been mentioned that much controversy has already arisen among workers on the cytology of the Cyanophyceae over the question whether or not these organisms possess a nucleus. The answer to this question must necessarily depend upon the standard of judgment. If we use as a standard the well differentiated structure in the flowering plants known as the nucleus, with its well defined membrane separating it from the surrounding cytoplasm while in a resting condition, and with its intricate method of division, whereby its most highly organized substance, the chromatin, is handed down to the daughter nuclei in exactly equal quantities, then there is no nucleus in any one of the forms upon which I have thus far worked. This criterion, if rigidly held to, would eliminate a number of structures which have hitherto been considered as nuclei in the lower forms of life, and even in certain cells of highly organized animals and plants. But if it be conceded that these structures last referred to are nuclei, then there is a nucleus in the Cyanophyceae, clearly and unmistakably. The writer's conclusion is based upon the following evidence. First, the appearance of the nucleus in the living cell; second, its reaction to stains; and third, its chemical nature. There is present in the center of the cell of the Cyanophyceae a small refractive structure, well defined in some species and much less so in others, and quite constant in outline in a given species. In order to detect the presence of nuclei in doubtful cases and to make a more careful study of the parts and their behavior, staining is necessary. By this treatment it is found that certain parts,

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particularly during cell division, uniformly have a stronger avidity for stain than other parts, and that these parts have a definite procedure during every cell division. It has been found that iron and phosphorus are constant constituents of the nucleus. For the detection of these elements in the nucleus of the Cyanophyceae I have relied upon the careful investigation of Macallum, who has demonstrated the presence of both masked iron and organic phosphorus. These lines of evidences are corroborative. The refractive body is not a homogeneous mass, but contains a portion which has a strong affinity for "basic" stains, and contains masked iron and organic phosphorus. This structure is present as a constant organ in all living cells of the Cyanophyceae, divides at each cell division, and, so far as can be judged with present knowledge at hand, it performs all the functions of a cell-nucleus. In the mind of the writer this evidence seems ample to warrant its being called a nucleus, and it will be treated as such in the remainder of this account.

The nucleus of the Cyanophyceae cell contains, in all of the healthy vegetative cells of the forms studied, a ground mass, granules, and chromatin.

The ground mass of the nucleus has not been a subject of special investigation in this study, and the writer is not prepared to say much concerning the finer details of its structure and the part it plays in the life phenomena of the cell, but from what has been seen it seems to play no essential rôle in nuclear division except to act as a matrix in which the other parts are at all times embedded.

The granules vary in size and number. There may be one or more in each nucleus, according to the species and the conditions. Of this more will be said under the head of granules.

In any group of organisms in which there is a diversity of morphological differences such as exist in the Cyanophyceae, one would not expect to find the same nuclear differentiation throughout the group. The variety of nuclear forms found in the groups helps to confirm the universal belief in organic evolution, for among the various members of the group we find instead of a single pattern of nucleus a number of nuclear forms which may merge into each other, but which indicate divergence from a com-

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mon type. It is the belief of the writer that when all the species of the Cyanophyceae are investigated cytologically, a complete and intelligible series of nuclear differentiations with but few, if any, gaps will be discovered, beginning with a very simple form, and finally diverging into several types which will be found to join on to various species of Chlorophyceae by almost imperceptible degrees, thus closing up the gap which seems to exist between these two groups of plants.

While it is not the intention to attempt in this paper anything like a phylogenetic arrangement of the species of the group, a sufficient number and variety of forms have been worked upon to note the existence of at least three quite well defined types of nuclei. Furthermore, a sufficient number of forms have been worked out to warrant the conclusion that we have in this group of plants a series of nuclear modifications showing how the nucleus of the higher plants may have originated. These three types are based upon the degrees of differentiation of the chromatin, and its behavior during cell division. They are here designated as the Diffuse type, Net-karyosome type, and Primitive Mitosis type. The third type may be considered as having arisen from the first by a slight modification.

Judging from the species I have worked upon, and the morphological relation of these to other species in the group, the diffuse type seems to predominate, and probably represents the most primitive nucleus in the group. We will turn to its consideration first. This type is characterized by having the chromatin distributed throughout the nucleus in an indefinite way, in the form of thin plate-like or small angular pieces, or more or less branched and knotty thread-like masses, according to the species and to the shape and size of the cell in which it is located; or even a combination of these may be found in the same species. In whatever shape the chromatin is found, the essential characteristic is its quite equal distribution throughout the nucleus. It is to be found in the homocysted group, *e.g.,* in *Oscillatoria, Lyngbya, Phormidium, Symploca,* etc. Within this type may be found quite a continuous series, showing how a very primitive nucleus becomes modified by gradual steps in the direction of the nuclear structure found in the higher plants.

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The lines of modification are, first, the concentration of the whole nucleus toward the center of the cell and the consequent concentration of the chromatin; and, second, the union of the chromatin into a more or less continuous thread. The differentiation seems to be proceeding toward the spireme formation found in the dividing of the nucleus of the higher plants. Of all the species of Cyanophyceae thus far worked upon, Fig. 1 represents the type in its simplest form. In this species the nucleus occupies the larger part of the cell, and the chromatin is distributed throughout it in the form of disconnected irregular pieces. Fig. 2 represents a modification of this in the direction of the union of the chromatin into more of a continuous mass. Fig. 3 represents a modification in the concentration of the nucleus toward the center of the cell. In Fig. 4 we see both greater concentration of the nucleus and greater union of the chromatin. The nucleus in Fig. 5 represents about the extreme in both directions. It occupies a relatively small amount of space in the cell and is usually, though not always, united into a single mass, much branched, with free ends projecting. There may be smaller free masses in addition to the large mass, which is not solid throughout. Fig. 6 represents a nucleus with very irregular outline in which the chromatin is in the form of thin irregular plates, mostly united around the margin but discontinuous in the middle.

Longitudinal views of Figs. 1, 3, 4, and 5 are represented by Figs. 7A, 8, 10, and 9 respectively.

These figures are all of comparatively short-celled species; that is, relatively short as compared with the diameter of the trichome. The cell of Fig. 1 is about 30μ in diameter and the cells are from 3μ to 5μ long. In species whose cells are several times longer than broad the nucleus is extended in the longitudinal direction, and has a tendency to arrangement of the chromatin into the form of threads somewhat united, and these are oriented more or less parallel to each other, and parallel to the long axis of the cell. Figs. 12, 13, and 17 represent such an arrangement.

Figs. 11, 24, 25, and 26 represent a still higher degree of differentiation in the direction of a sharper separation of the cytoplasm and the nucleus. The chromatin is collected near the center

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of the nucleus into a few thread-like masses. In these respects it more nearly approaches the nucleus of higher plants than any other form studied. This is the only species of this genus which I was able to differentiate so sharply. Not every collection of this species showed the same structure. This indicates the breaking up of the type into several species, some of which are more highly differentiated than others. The measurements given for the species cover a greater range than one finds in a single collection. This case illustrates how some of the discrepancies may have arisen in the accounts of workers on the group.

The second type, the Net-karyosome type, is quite rare. I have come across but two well marked cases so far. These are found in the genus Dermocarpa, two species of which I have worked upon, *viz., D. fucicola* and *D. prasina,* the former of which is represented by Fig. 33. This type, with perhaps some modifications, will probably be found to be characteristic of all the forms of Chamaesiphonaceae, which reproduce in a similar manner to *Dermocarpa.* The type is characterized by having the chromatin united into a very definite fine network on which, particularly at the junction of the threads, are small granules or knots, presumably of chromatin, since they stain like the remainder of the thread. This whole network occupies a very large part of the cell, leaving only a narrow zone outside next to the cell-wall. Material of *Dermocarpa* killed in Flemming's mixture and sectioned does not differentiate satisfactorily with the nuclear stains. Aqueous methylene blue and Ehrlich's haematoxylin yielded fine results on material killed in potassium iodide-iodine.

The spores of *Cylindrospermum* have the chromatin arranged in very nearly the same manner as it is in *Dermocarpa.* I have not come across any forms yet in which there are granules on the thread, and the thread is not formed into quite so definite a network as in *Dermocarpa.* Figs. 19, 20, and 21 show the condition of the chromatin in the spore of *Cylindrospermum.* It seems that in the genus *Cylindrospermum* we have a transition between the first and second types, the nucleus in the vegetative cells on one side approaching the first type and on the other side that in the spores approaching the second type.

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Only a slight modification of the first type is necessary to produce the third or Primitive Mitosis type, which has been found in a single species only (Figs. 40–44). In this type the chromatin unites into a single contorted thread quite irregular and indefinite in shape. This thread breaks into a definite number of pieces preparatory to cell division.

The foregoing description of types of nuclei applies to the resting condition.

It seems appropriate here to say a few words about resting nuclei, since Kohl and Olive do not consider that the nucleus ever assumes that condition. I am not sure that I understand their point of view on this question, but if their meaning is a condition of the nucleus in which the chromatin is distributed through the nucleus in the form of fine granules when not dividing as is generally the case in higher organisms, then I can agree that the nucleus of the Cyanophyceae never reaches a resting condition. But if a resting condition in the ordinary sense is meant, in which there is no visible sign of any nuclear division or cell-wall formation, then the nucleus of the *Cyanophyceae* is as often in a resting condition as the nucleus of any other organism. Olive speaks of centers of activity of cell-division occurring with rhythmic regularity in the trichomes of filamentous forms. This would indicate that the cells between these centers of activity must be in a resting condition. Under ordinary circumstances almost any species shows from 30 per cent. to 40 per cent. of the cells in which there is absolutely no sign of cell-division or change in the shape of the nucleus to indicate preparation for division, and I have had cultures dry up slowly in which at least 90 per cent. of the cells were in a quiescent state. When cells are dividing rapidly the chromatin will be found in many cells to extend from cross-wall to cross-wall, while in the same collection, and even in the same plant, one may find the chromatin in other cells concentrated near the middle of the cell with a layer of protoplasm between it and the cross-wall. Many of the heterocysted group produce spores which rest for a time, often for months. The homocysted group produces hormogonia which act in the same manner. These are simply small groups of cells, from two to several, which survive when a filament breaks up from any cause.

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These, like the spores, rest for a time until favorable conditions arrive, such as proper temperature, moisture, food, etc., then they resume growth and build up new trichomes. During this time they must be quite at rest.

(*a*) THE DIVIDING NUCLEUS.

The division of the nucleus in the Diffuse type is unmistakably amitotic. Whether in the short-celled form represented by *Oscillatoria margaritifera* (Fig. 7) or in a long narrow-celled form represented by *Oscillatoria splendida* (Fig. 17), the process is very much the same and quite simple. The chromatin is distributed quite uniformly throughout the nucleus in the resting cell. It presents no peculiar characters or modifications in form or structure during cell division that do not exist in the resting cell with the exception in many cases of a constriction in the middle as if the ingrowing cell-wall were actually pressing the chromatin toward the center before dividing it. It is impossible to say at present what causes the chromatin to separate. The separation always takes place at the outer margins first and proceeds toward the center of the cell. In no case have I noticed the whole mass separating simultaneously. Whether the ingrowing cell-wall plays any rôle in the process or not, it is never very far from the edge of the divided chromatin. Fig. 7 represents cells in various stages of division: *a* represents a resting cell, *b* one in which the division has proceeded only a short distance, *c* one almost divided, and *d* a complete division. The chromatin in these cells is represented in greater abundance than exists in the greater number of cells in this species. An instructive series is shown in Fig. 12, in which *a* represents the first stage in the series. The only sign of division is the beginning of the laying down of the ring-shaped cross-wall. No evidences of nuclear division are yet manifest: *b* shows the wall grown into the chromatin which is beginning to separate; *c* represents a stage in which the entire mass of chromatin has separated except a single strand in the center. In Fig. 18 may also be seen the same condition of chromatin

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division and ingrowing cell-wall. These are not rare cases, but may be found in great abundance in any collection of actively growing plants.

The division of the nucleus in all the filamentous species studied, including both homocysted and heterocysted forms, is direct, or amitotic. I have never observed in any such species the breaking up of the chromatin into a definite or an indefinite number of chromosomes either in the form of rods or of granules. After cell division the chromatin in each daughter cell (practically one-half of the original amount in each cell) undergoes no essential change, retaining the same general structure as before division, and simply increasing in quantity; in many cases it does not wait to attain its normal size before beginning another or even two divisions. Although the chromatin of the spores of various heterocysted species unites into network in a similar manner as does that of the Net-karyosome type, its division is the same as that of the vegetative cells, that is amitotic. The whole mass of network is divided into two equal parts, the separation beginning at the margin and proceeding toward the center until the whole is divided. Fig. 20 represents a nucleus completely divided and the cell-wall grown across, dividing the original cell into two equal parts. Spore division does not always take place in this manner. Usually several divisions may be seen in progress at the same time.

The division of the nucleus in the Net-karyosome type may also be said to be amitotic. No spireme, spindle, or chromosome formation is present. We have in the species in which this form of nucleus is present a different procedure in division from that which is usually found in other organisms. The mature cell of the *Dermocarpa* may be several hundred times larger than the original young cell; at the same time there is but one mass of chromatin all united into a definite network, or in other words, a single very large nucleus. The chromatin in this large network breaks up simultaneously into a large number of small parts about equal in size. These become the centers of new cells which are now formed by the simultaneous laying down of new cell-walls around each mass of chromatin and its surrounding cell-substance. The mother cell-wall now ruptures and the whole

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mass of small cells floats out. These cells are known as conidia or schizospores. They are non-motile, and when they fall on a suitable substratum, usually on some living plant, they begin to grow and the chromatin mass increases in size, very soon forming another chromatin net as before.

Of all the forms of nucleus in the Cyanophyceae which I have investigated the Primitive Mitosis type as illustrated by *Synechocystis aquatilis* (Figs. 40–44) approaches nearest to the mitosis of the lower Chlorophyceae. The chromatin unites into a thread in preparation for cell division. This thread is not, however, a very definite one as regards its shape and disposition in the cell. The thread breaks crosswise into a definite number of segments, in this species three, which arrange themselves parallel to each other and to the long diameter of the cell. These break apart in the middle when the new cross-wall is to be laid down.

In this method of division, as in that of the first method mentioned, the cell-wall seems to push the segments toward the center in some cases and they are not always divided simultaneously. Fig. 43 illustrates this method. It should be said that the division of the chromatin thread seems to precede somewhat the ingrowing of the cell-wall, and the impression is not produced, as it is in *Oscillatoria,* that the ingrowing wall simply cuts the chromatin mass in two.

It will be seen from the foregoing methods of division of chromatin in the Cyanophyceae that there is not an exact division of the chromatin into equal parts, but only an approximation thereto, and further that there is no longitudinal splitting of the threads of the chromatin.

The formation of a constant number of chromosomes and the longitudinal splitting of the same in order to accomplish the exact partition of all of the chromatic elements, the bearers of the hereditary qualities, have constituted a working hypothesis upon which to explain inheritance. It is acknowledged that these plants have been handed down to us from very ancient times, and yet without this complicated karyokinetic process found in higher organisms they go on reproducing themselves in such fashion that each succeeding generation resembles the parent one as nearly as may be found elsewhere in the organic kingdom.

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(*b*) THE GRANULES.

The granular inclusions of the Cyanophyceae cell have occasioned much controversy. The questions of dispute have related to their number in a cell, their constancy, their location, their chemical composition, their function, and the number of kinds.

With the writer they have not been a subject of thorough investigation, yet some things have been quite definitely determined about them and these are recorded here. The investigation has led to the discovery of but two kinds of granules in the Cyanophyceae cell. These are doubtless the same that have been seen by many writers, but no attempt will be made here to try to harmonize the writer's discoveries with those of other workers, for it would probably only lead to further confusion of an already tangled subject. A number of different names have been employed to designate these various kinds of granules, some relating to their location, some to their reaction to stains, and some to their chemical composition and function. I am not at present prepared to say what terms should be used to designate these two sorts of structures which I have differentiated, and for convenience in this paper will employ the terms α and β for this purpose.

My conclusion that there are two kinds of granules present is based mainly upon their behavior toward stains. No chemical tests have been made. Results obtained by straining are at times deceptive, and should be supplemented by other methods. In the study of the granules the writer proceeded in a similar manner as in the study of the nucleus, finding out as much as possible with the living material, then by the application of concentrated aqueous stains to the living material. About all one can discover in the living material is that in certain species granules are present and some of them are arranged in quite a definite way, *e.g.*, along the cross-walls. In other species granules may be seen in the center of the cell, but in neither of these cases can one always see them in these locations, and further, if a species has them located in both positions, one is not able to say where the dividing line is, since they look very much alike in the living cell. To segregate them it was necessary to employ some stain that would

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stain one kind and not the other; and equally important, one that would not stain the other cell structures to obscure the granules. Two stains were found that will do this unerringly in all the species worked upon. These are Bismarck brown and wasserblau.

One kind of granules, which for convenience will be called *a*, are located within the nucleus in every healthy living cell of each species studied, with perhaps a few exceptions among the unicellular forms and in the genus *Merismopedia* (which were investigated at the beginning before proper technique was employed, and which I have not since been able to obtain). There is another exception which I have not yet been able to explain satisfactorily, *viz.*, mature spores.

The location of these granules is variable and depends upon the shape and size of the cell and also upon the structure of the nucleus. They are always in close proximity to the chromatin, but not imbedded in it, even in small filamentous species where the chromatin is often in a single irregularly shaped mass (Fig. 38). In the large species of *Oscillatoria* and other large filamentous forms they are scattered promiscuously through the nucleus among the chromatin elements.

The number of these granules is also variable in the same species at different times and in different species. The variation is probably due to differences in cell activity. In resting cells there is a tendency toward a constant number. At least this seems to be the case in small-celled species in which one can easily count them. There are often filaments of *Lyngbya Lagerheimii* (Fig. 38) in which there is but a single large granule close to the chromatin mass in each cell throughout almost the entire filament. These *a* granules occur sometimes with such regularity as to lead one to suppose them to be nucleoli, but they do not behave like nucleoli of higher plants, which tend to disappear during cell division. On the contrary, they seem to increase in number when the cells are actively dividing.

The exception above spoken of, *i.e.*, that they do not occur in the spores, seems significant. This was a difficult point to determine since the spores are not easy to stain on account of their thick walls, yet some stains were found which penetrate the wall.

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Ehrlich's haematoxylin is excellent for this purpose, since it does not stain anything deeply except the granules (when they are present in the young spores) and the chromatin. It has been found by the use of this stain that these granules disappear before the spore reaches maturity. They do not disappear at once, but become gradually smaller and finally disappear entirely. These granules arise *de novo* in the cell, and are not handed down by division and subsequent growth. It so happens, however, that if there are but a few in a cell they are so oriented that some will get into each daughter cell, for no healthy vegetative cell is without them.

I have at times seen appearances which indicate that the α granules divide, but the evidence is not sufficient and conclusive. There often appears to be a connection between two granules, but I presume that this is simply the deeply stained protoplasm the color of which is not washed out. The new granules usually arise in close proximity to the older ones. One may often see a series of several sizes together ranging quite gradually from largest to smallest. *Cylindrospermum* is an excellent genus in which to study them in the vegetative cells, and *Anabaena variabilis* furnishes a good example of their disappearance during spore formation, for here the spores mature gradually in a series.

Many of the granules appear hollow, or at least only the outer rim stains. These are usually the larger ones, the smaller ones appearing to be solid. The stain which I have so far found to be unerring in differentiating them is a saturated aqueous solution of Bismarck brown used on living material. This makes it exceedingly quick and simple to examine a species to detect if they are present or absent. Living material mounted on the slide as previously described may be stained, dehydrated and mounted in balsam within a short time, varying from a few minutes to half an hour, according to the species, but there is scarcely ever any danger of over-staining. The granules of material killed in potassium iodide-iodine or in alcohol stain equally as well with Bismarck brown as the living material. In some species the granules may become swollen when stained. Besides Bismarck brown, there are other stains which will stain equally well and differentiate these granules in some species. Methylene blue in aqueous

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solution on the living material is very fine for this purpose, but in many species there are too many other things stained at the same time for one to identify these granules positively. Usually the chromatin is stained blue and the granules red with this stain. An aqueous solution of thionin is also very satisfactory for staining the α granules. It is necessary to over-stain and then wash out with acidulated alcohol. The granules will be the last to disappear and these will be stained red. Fig. 14 represents a cell of *Oscillatoria margaritifera*, killed in potassium iodide-iodine and treated as above described.

The other, or β granules, are readily demonstrated with aqueous solution of wasserblau. The cytoplasm will also take up the stain and obscure the granules, so in order to differentiate them it is necessary to over-stain and wash out with acidulated alcohol, since alcohol alone will not sufficiently dissolve the stain to reveal them. They will be the last to disappear and will stain deep blue. Eosin and picrocarmine will also stain them, but not so satisfactorily.

Unlike the α granules (which are always present), the β granules may be or may not be present in a given species, although in some species they are quite constant. In some collections all of the plants will possess them, in others only certain plants, and still others only certain cells in a filament. Their presence seems to be due to physiological conditions, and in certain cases they seem to have quite a definite relation to the α granules. Thus in a spore formation in the heterocysted group, as the α granules disappear the β granules increase in number and size. There are indications of this phenomenon in the homocysted group. The β granules seem to increase as the plants go into a resting condition and the α granules seem to diminish. However, my observations on this last point are not sufficient to form a basis for judgment in the matter, but in the case of the spores it is true that the β granules increase greatly in number and somewhat in size (at times almost completely filling up the spore), while the α granules disappear entirely. The α granules probably give up their material either to form chromatin or to form the β granules, and the former is more likely since they are

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so closely united to the chromatin, while the β granules are probably accumulated food material.

In the homocysteae the β granules are located most frequently along the cross-cell walls, the location, however, being a specific character. In some species they are distributed quite evenly throughout the cytoplasm, and in a single instance (*Symploca Muscorum,* Fig. 13) they are scattered among the α granules in the nucleus, at least wasserblau which invariably differentiates them in other species stains some granules blue within the nucleus.

They arise *de novo.* Their development may be watched in *Lyngbya aestuarii* (Fig. 35), in which they are arranged along the cross-walls only. As the new wall grows in from the side, soon the β granules appear. They increase in size and number as the cell grows older. Fig. 39 represents their arrangement and occurrence in *Oscillatoria splendida.* Fig. 15 represents them in end view of *Oscillatoria margaritifera* stained with wasserblau, and Fig. 14 represents the first species first stained with thionin, then counterstained with wasserblau. The α granules are red and the β granules are blue. They are both represented in the same plane, but the α granules are, in end view, below the β granules; that is, they are within the nucleus while the β granules are along close to the cross-cell wall. This same differentiation is shown in Fig. 13, in which case the α granules are stained red with Ehrlich's haematoxylin.

(*c*) THE CYTOPLASM.

The space between the nucleus and the cell-wall is occupied by the cytoplasm, the structure of which, as would be expected, varies considerably in its finer details in different species, and also in different cells of the same species.

Much controversy has arisen over the shape of the achromatic portion of the nucleus. Under a certain treatment in some species it seems to extend as a distinct substance out into the general cytoplasm by means of fine radiating arms. My investigation has not yet been conclusive on this point. The stains which best differentiate the chromatin do not differentiate the achromatic por-

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tion distinctly, and no counterstain has yet been found that will do this, so I withhold judgment on this point at present.

In young cells, and in species in which no β granules are present, the cytoplasm, when killed with Flemming's solution and stained with Heidenhain's iron haematoxylin, is shown to consist of a regular network of delicate threads. In other species in which there is an abundance of β granules present, under the above treatment the cytoplasm has the appearance of a definite alveolar structure, because the granules do not stain with the haematoxylin.

There is usually a thin layer next to the cell-wall which in all cases is much finer in structure than the coarser structure toward the center. Figs. 1 to 6 inclusive show the protoplasmic structure in a plane in which no granules are present.

I have not been able to demonstrate protoplasmic continuity as seen by other workers. I have tried a large series of distinctively plasma stains for this purpose without meeting with success. Ziel's carbol fuchsin is a very energetic and selective stain, and leaves the cell-wall transparent, while the cytoplasm is deeply stained; yet in no species studied, from the smallest to the largest, have I been able to detect any connective strands of cytoplasm between the cells except in case of dividing cells, in which the ingrowing cross-walls have not yet completely separated the cells.

The connection between the heterocyst and the vegetative cell or spore may easily be demonstrated with aqueous solution of wasserblau on living material, washed out with acidulated alcohol after over-staining.

The cytoplasm contains the coloring matters, there being no special differentiation of chromatophore for this purpose.

IV. THE PRODUCTS OF ASSIMILATION.

A few tests were made to ascertain what are the products of assimilation. Starch has not been found in any of the species studied. Some authorities state that glycogen is a constant constituent of the Cyanophyceae cell as the first product of assimilation, while others affirm that it is never present in these plants.

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The ordinary microchemical tests did not yield definite and conclusive results to the writer. In order to test the matter further, the method of extracting glycogen from animal tissue was resorted to. Mercuric chloride and Esbach's reagent were used to precipitate proteids in macerated rabbit's liver, after which the glycogen was precipitated with alcohol. This was done to test the reagents and to secure glycogen for comparison. A quantity of *Oscillatoria* was then rubbed up in the same way with some of these same solutions, and filtered. The filtrate was then treated with alcohol, and a precipitate was thrown down which had the same appearance as the glycogen prepared from the rabbit's liver, and which gave all of the characteristic glycogen reactions. From this result it seems just to conclude that glycogen is produced in the Cyanophyceae.

Before making this test for glycogen, I was of the opinion that it is not to be found in this group of organisms, but that sugar is the first product of assimilation, and that the excess is stored in this form. I came to this conclusion because aqueous extracts of a variety of forms of Cyanophyceae pulverized with fine sand in a mortar always readily reduced Fehling's solution.

It is of course possible that this reducing body is something else than sugar; but, since glycogen is produced in the animal body from sugar, it is quite probable that the first product of assimilation in the Cyanophyceae is sugar, and that some of this is converted into glycogen and stored in this form.

V. EXPERIMENTAL CULTURES.

I have already mentioned that the Cyanophyceae as a group have persisted doubtless from very ancient time, because of their power of adaptability to changes of environment without much modification in form. There is scarcely a habitat to which some species has not adapted itself, and in many cases the same species is capable of withstanding extremes in heat, moisture, and air supply.

Several writers on the group have stated that change in habitat, even though slight, results in profound changes in cytological characters. The writer has made some observations and per-

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formed some experiments with the view of ascertaining if possible the nature of these changes. The effect of being subjected to long drought was first studied. A collection of *Phormidium uncinatum* was made after it had been dry for five months. The material was wetted and examined in this condition; afterward the aid of stains and fixing agents was resorted to. The results showed that what I have since proved by experimentation, *viz.,* that a large percentage of the cells were in a resting condition brought about by slow desiccation. A large number of hormogonia had been formed, and these began to grow very rapidly, soon crawling out of their sheaths. After a few days, more of the material was stained, and the only change that had taken place was the appearance of rapidly dividing cells. The chromatin, instead of being drawn into the center of the cell, in many cases now extended through a series of partially divided cells, or, in case of a recently divided cell, it was still found close to the cross-wall. So this sudden change from an aerial dry condition in the direct sunlight (in which it had persisted for months) to submergence in water and diffused light does not produce any perceptible change in the structure of the cell contents nor in the character of their reaction to stains.

Still further to test the effect of change from an aerial to an aqueous medium, living material of *Oscillatoria sancta,* found actively growing on soil in a greenhouse, was placed in water. In this instance there was not only a change from an aerial to an aqueous environment, but also a sudden change in temperature. The growth of the plants was not interfered with to a noticeable degree, and the cytological characters remained unaltered. The normal habitat of this species is of course not in a greenhouse, but outside, where it is subjected to variation of light, heat, and moisture. The same structure of the cell appears when these plants from a normal habitat are stained.

Symploca Muscorum withstood being dried up several times without manifesting any cytological modifications. A change in habit of growth occurs when this species is changed from an aerial to an aqueous environment. In the air the filaments unite into fascicles, somewhat cone-shaped, but in water they spread out.

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The effect of changing the concentration of the liquid medium was tried. *Lyngbya aestuarii* growing in salt water was transferred from this into fresh water, also into distilled water. Material was studied before and after the change, and on comparison the structure of the protoplast presented no unusual modification. This species will survive the change from salt to fresh water for many months, and even a surprisingly long time when changed to distilled water.

The rate of growth may be influenced in various ways. Other things being equal, the amount of light is a prominent factor in influencing cell division. If young, actively growing material be placed in the dark, growth proceeds very slowly, and after a few days or weeks, depending upon the species, will discontinue almost entirely. In diffused light growth proceeds more rapidly than in the dark, and in direct sunlight the maximum growth takes place. This is doubtless due in a large measure to nutrition rather than to the direct action of light rays. As a continuous habitat, however, some species prefer diffused light rather than direct sunlight. The comparative activity of cultures placed in direct sunlight and of those placed in diffused light or in the dark may usually be detected within a few minutes. Those placed in direct sunlight will be seen to be giving off more oxygen than the others. This collects and forms bubbles, which become entangled and held fast in the mass of filaments, in many cases soon buoying them up to the surface of the water. This has already been alluded to under methods of obtaining clean material. The metabolism of those in diffused light or in darkness is usually not sufficiently rapid to cause the accumulation of gas, which is absorbed by the water as fast as given off.

It is quite remarkable how tenacious of life some forms are when placed in the dark. A culture of *Aphanothece* which was in fair vegetative condition was placed in a dark chamber and supplied with enough water to keep it moist for sixty days. It then dried up and remained dry and brittle for fifteen more days. It was again wetted and some of the material stained, when it was found that a few cells were still dividing and that very few had died. The nucleus had become somewhat more concentrated into the center of the cell, and there were fewer gran-

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ules. The color was more of a "grass green" than a "blue green," which seems to indicate that the phycocyanin is first used up or disappears first under these conditions. This same material was then placed in the light and kept moist. It assumed its original color again, and grew for three months afterward.

To induce a resting condition in the cell, I have succeeded best by placing healthy material on compact wet earth and covering the vessel in which it was placed with glass in such a way as to allow evaporation to take place slowly until the material was completely dry and brittle. On wetting this material again and applying stains, it was found that at least 90 per cent. of the cells were in a resting condition, and that the nuclei were a little more concentrated in the center of the cell than they were in the actively growing plants, although otherwise the cell organs appeared to be the same as before.

VI. THE RELATION OF CYANOPHYCEAE TO BACTERIA.

The relation of the Cyanophyceae to the Bacteria, particularly to the sulphur bacteria, has been a subject of research by a number of investigators. Among those who have given special attention to the cytology of these organisms are Bütschli, Fischer, and Macallum.

In *Beggiatoa,* Bütschli was able to demonstrate a deeply staining part in the center of the cell and a lighter staining peripheral portion. In *B. alba* this peripheral portion he found to be very narrow. He looks upon the central portion as the analogue of a nucleus. Fischer and Macallum did not succeed in bringing out this differentiation. Macallum demonstrated the presence of "masked" iron and organic phosphorus uniformly distributed throughout the protoplast of *B. alba* and *B. mirabilis.*

The writer has given but little attention to these organisms, having worked upon but a few forms of the sulphur bacteria, chiefly of the genus *Beggiatoa.* These forms are usually so filled with sulphur granules that it is difficult to study the structure of the protoplast while these are present. They may readily be re-

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moved with alcohol, however, without seriously injuring the cell structure, if the material is previously killed in potasium iodide-iodine or in Flemming's solution. Figs. 28 and 29 respectively represent optical longitudinal and cross sections of *Beggiatoa arachnoidea,* killed in Flemming's solution, dehydrated in alcohol until all of the sulphur was dissolved out, and then stained with Loeffler's methylene blue. By this treatment the protoplast is seen to consist of a regular coarse network uniform throughout the cell, with the exception of a very narrow zone next to the cell-wall. This seems to indicate a slight differentiation of the protoplast in the direction of the simplest differentiation found in the Cyanophyceae, such as represented by Fig. 1. Iron haematoxylin and some other chromatin stains differentiate the cell structures in the same manner. As to the differentiation into two zones, my results correspond quite closely to Bütschli's interpretation of the structure of *Beggiatoa alba,* with the difference, however, that in my preparations the "central body" does not appear homogeneous, but consists of a network which occasionally extends to the cell membrane. I am not prepared to say whether this network contains chromatin or not. It seems quite probable that it does contain chromatin, however, for Macallum has shown that iron and phosphorus, the essential elements of chromatin, are distributed throughout the cell. Should it prove to be true that this network does contain chromatin, then the coarse network could well be looked upon as the analogue of a nucleus, and in this species only a slight differentiation has taken place between it and the peripheral cytoplasm. There is not a very great gap between this condition and the one found in the differentiation in *Oscillatoria margaritifera.* Other forms will doubtless be found in which the difference in structure will be even less than the difference between these two species.

In *Beggiatoa mirabilis* there are in every cell a few granules which react in the same way to stains as the α granules in the nucleus of the Cyanophyceae, and these are not affected by the stains which color the β granules found in the cytoplasm of the Cyanophyceae. There is undoubtedly a close genetic connection between the *Beggiatoas* and the homocysted forms of Cyanophyceae, particularly the *Oscillatorias.*

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VII. RESULTS.

1. The cell of the Cyanophyceae contains a nucleus which in some species is sharply delimited from the surrounding cytoplasm, while in others the differentiation is much less marked.

2. The nucleus occupies a relatively large portion of the cell, its shape being determined by the shape of the cell. In short-celled filamentous species its greater diameter is across the filament, while in long-celled species it extends in the direction of the long diameter of the cell.

3. In all the species studied, with the possible exception of *Synechocystis,* the nucleus divides amitotically, beginning at the periphery and gradually proceeding to the center. In *Synechocystis aquatilis* a primitive form of mitosis has developed, in which there is a spireme formed which separates into three pieces. These arrange themselves parallel to each other and to the long diameter of the cell, after which they are divided crosswise, and the ingrowing cell-wall completes the cell division. No longitudinal splitting of the thread occurs.

4. The nucleus consists of granules, chromatin, and an achromatic ground substance in which the two former substances are imbedded. In some forms (*e.g.*, the large short-celled species of *Oscillatoria*) the chromatin is disposed in the ground substance in the form of disconnected masses; in others (*e.g., Symploca Muscorum*) it is somewhat united into a coarse thread-like mass; and in still other cases (*e.g., Dermocarpa*) the chromatin is united into a definite network.

5. The nucleus may be induced to assume a resting condition by very slow desiccation.

6. There is no definitely organized chromatophore, the cytoplasm holding the coloring matters.

7. Cell division is completed in the filamentous forms by the gradual ingrowing of the ring-shaped cell-wall. The division of the chromatin in some species seems to precede the ingrowing of the cell-wall; in others it accompanies it, beginning at the outside of the mass, and keeping pace with the ingrowing wall; and in still other species the cell-wall actually grows to and against the chromatin, gradually crowding it toward the center as if it

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were cutting it in two. In *Dermocarpa* the nucleus breaks up simultaneously into a large number of daughter nuclei; the process is amitotic.

8. Two kinds of granules have been demonstrated in the Cyanophyceae cell. One kind, designated as α granules, is located in the nucleus of the vegetative cell in close proximity to the chromatin (this kind is not present in the mature spore); the other kind, designated as β granules, may or may not be present in the vegetative cell, but is always present in the mature spore. The age of the plant, physiological conditions, and variation in nutrition are probably the determining agencies affecting the presence or absence of the β granules.

9. No protoplasmic continuity between the vegetative cells has been demonstrated.

10. Change in habitat does not produce any marked change in cytological characters.

11. One of the products of assimilation is glycogen. Sugar is probably one of the first products of assimilation.

VIII. SUMMARY.

A new type of nuclear division has been discovered in *Dermocarpa* in which the nucleus breaks up simultaneously into a large number of daughter nuclei by a process of amitosis. (See Figs. 33, 34.)

The present investigation reveals in the Cyanophyceae a series of nuclear structures, beginning with a very simple form of nucleus scarcely differentiated from the surrounding cytoplasm and dividing by simple direct division. From this we pass by very gradual steps to a highly differentiated form of nucleus which in dividing shows a primitive type of mitosis, and in structure approximates the nucleus of the Chlorophyceae and the higher plants. (See Figs. 28, 1 to 5, 40 to 44.)

In this group of plants the transmission of hereditary qualities seems to be accomplished with the greatest precision, without the complicated machinery of mitosis. In this connection it may be noted that the lack of sexuality seems in no wise to affect

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the amount of variation, which is quite the same as in groups where sexual reproduction occurs.

In conclusion I wish to acknowledge my gratitude to Prof. W. J. V. Osterhout, under whose direction the work has been performed, for many helpful suggestions; and to Prof. W. A. Setchell for the determination of the specimens considered in this paper.

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EXPLANATION OF PLATES.

K. I.—Potassium iodide-iodine.

Ehr. Haem.—Ehrlich's haematoxylin.

All of the colored figures represent optical sections and were drawn with the aid of the Abbé camera lucida, Zeiss. Apoc. Hom. Im. Obj. 2.00 mm., Comp. Ocs. 12 and 18.

PLATE 21.

Figs. 1 to 5 present a series showing progressive differentiation of the nucleus. Fig. 28 might be looked upon as the first member of the series.

Fig. 1. *Oscillatoria margaritifera.* Cross section. Ehr. Haem. The outer narrow layer of cytoplasm is denser and composed of a finer network than the portion of the cytoplasm between it and the nucleus. This is true of all the species figured on this plate. The chromatin is in disconnected masses.

Fig. 2. *Oscillatoria limosa.* Cross section. K. I. Ehr. Haem., counterstained with wasserblau 5 seconds. The chromatin is more abundant and more closely connected than in *O. margaritifera.*

Fig. 3. *Oscillatoria chalybea.* Cross section. K. I. Ehr. Haem., counterstained with wasserblau 5 seconds. The nucleus in this species is more concentrated toward the center of the cell than in Fig. 2, and the chromatin is in the form of fine threads, somewhat connected.

Fig. 4. *Oscillatoria Okeni.* Cross section. K. I. Ehr. Haem., counterstained with wasserblau. The nucleus occupies relatively about the same area of the cell as in Fig. 3, though it is more united into thicker masses.

Fig. 5. *Oscillatoria brevis* var. Cross section. K. I. Ehr. Haem. The nucleus is more concentrated than in any of the foregoing species. The chromatin is united into a single very coarse net-like mass.

Fig. 6. *Oscillatoria sancta.* Cross section. K. I. Aqueous thionin. The nucleus is very irregular in outline and the chromatin has a tendency to a radial arrangement.

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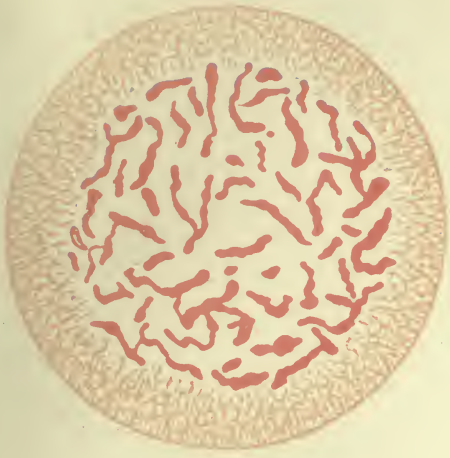

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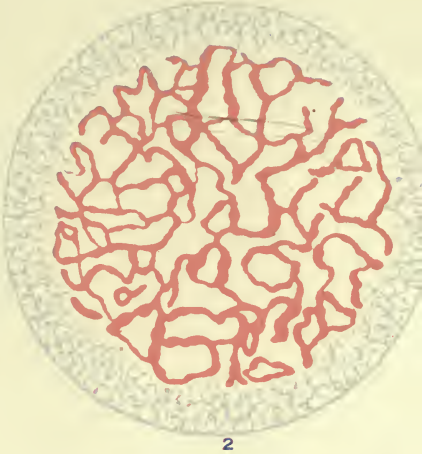

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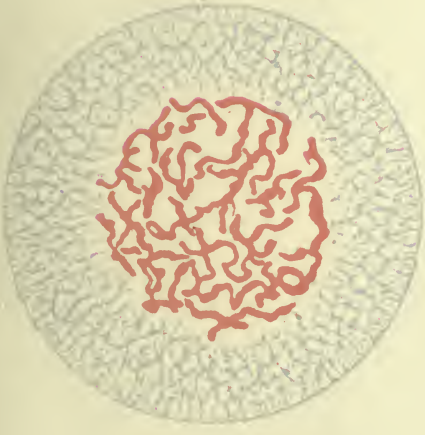

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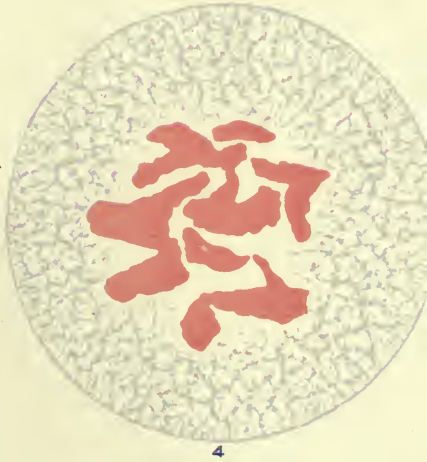

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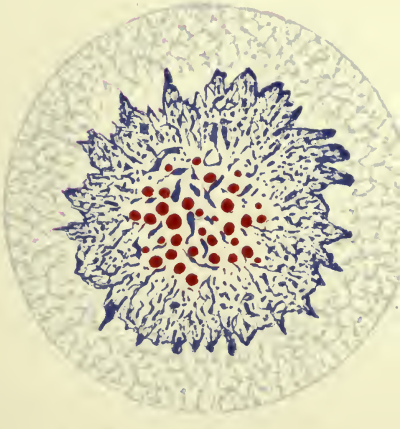

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PLATE 22.

Fig. 7. *Oscillatoria margaritifera.* Longitudinal section. K. I. Ehr. Haem. *a* represents a resting cell, *b* the beginning of cell division, *c* a cell almost divided, and *d* a recently divided cell.

Fig. 8. *Oscillatoria chalybea.* Longitudinal section. K. I. Ehr. Haem. This was stained longer than the preceding in order to bring out the *a* granules in the nucleus. The unstained quadrangular bodies have not been identified.

Fig. 9. *Oscillatoria brevis* var. Longitudinal section. K. I. Ehr. Haem.

Fig. 10. *Oscillatoria Okeni.* Longitudinal section. K. I. Ehr. Haem., counterstained with wasserblau. The cells are slightly shrunken and pulled apart, leaving the dividing nucleus in the sinus. The nucleus occupies nearly the entire length of the cell, and the chromatin has a tendency to arrange itself in the longitudinal direction of the filament.

Fig. 11. *Cylindrospermum licheniforme.* Longitudinal section. Living material stained with Ziel's carbol fuchsin, and counterstained with methylene blue. The first stain colors the cytoplasm and the chromatin red, and these are changed to blue by the second stain, which also stains the granules red. The two cells are slightly pulled apart, showing a strand of chromatin still undivided between the nearly divided cells.

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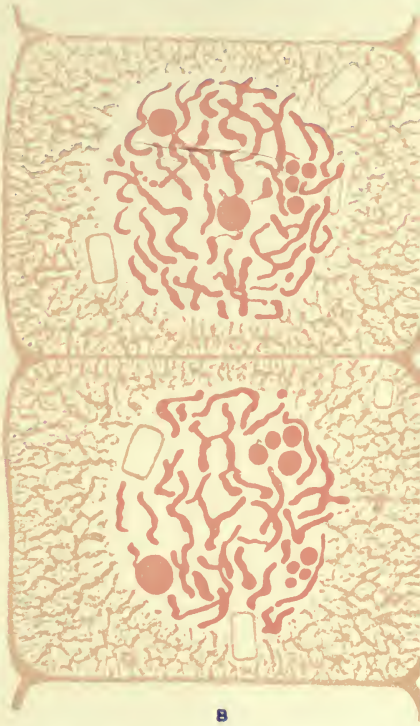

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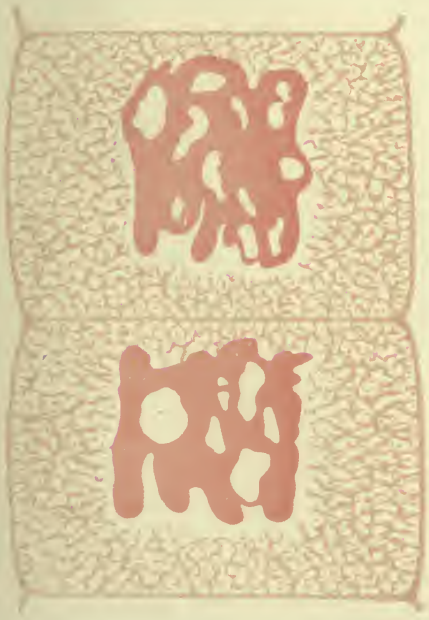

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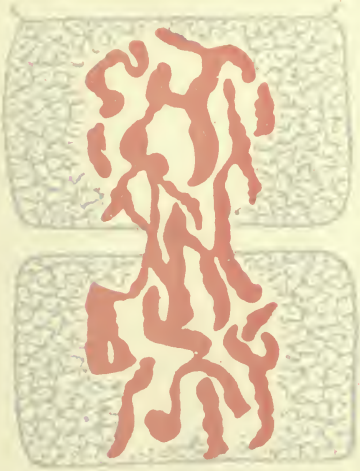

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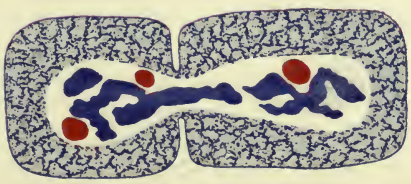

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PLATE 23.

Fig. 12. *Symploca Muscorum.* Longitudinal section. Living material stained with aqueous methylene blue. The cytoplasm has a greenish blue color, the chromatin a deep blue; the granules are red. *a* is a cell just beginning to divide; *b*, the cell-wall has grown into the chromatin, which is just beginning to separate; *c*, division almost completed.

Fig. 13. *Symploca Muscorum.* Longitudinal section. K. I. Ehr. Haem. 30 minutes, counterstained with wasserblau 5 minutes, and washed out with acidulated alcohol, then with Ehr. Haem. again. Wasserblau reveals the β granules in the nucleus and in the cytoplasm.

Fig. 14. *Oscillatoria margaritifera.* Cross section. K. I. Aqueous thionin washed in acidulated alcohol, counterstained with wasserblau and again washed with acidulated alcohol. The α granules are stained red and the β granules are stained blue. They are both here represented in the same plane, but in reality the red ones are in a plane below the blue ones.

Fig. 15. Same species and view as Fig. 14. K. I. Wasserblau washed in acidulated alcohol. Shows only β granules.

Fig. 16. Same species and view as Figs. 14 and 15. K. I. Aqueous thionin washed in acidulated alcohol.

Fig. 17. *Oscillatoria splendida.* Longitudinal section. Living material Ehr. Haem. 5 minutes, counterstained with wasserblau 15 seconds. Ehr. Haem. was not on long enough to stain the α granules, nor was the wasserblau on long enough to stain the β granules.

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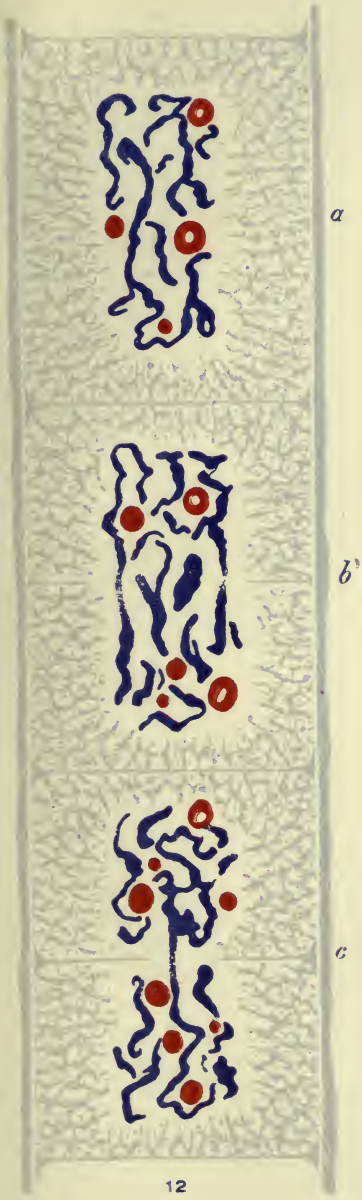

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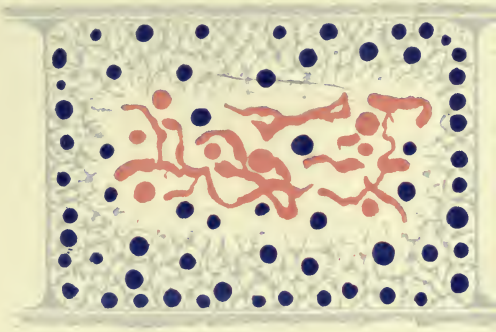

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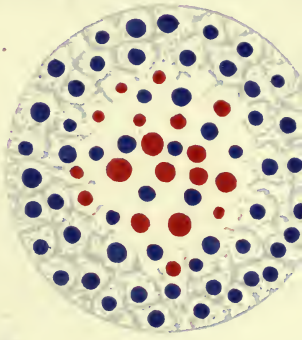

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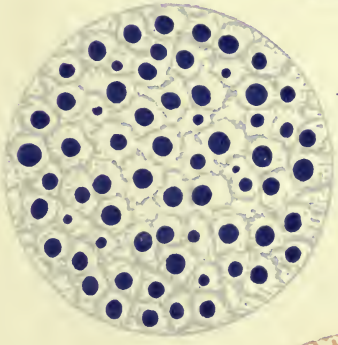

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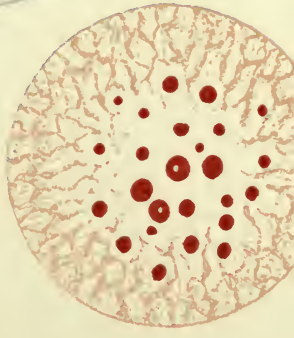

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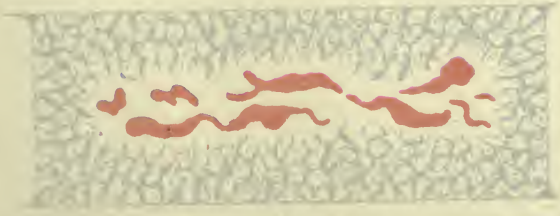

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PLATE 24.

Fig. 18. *Cylindrospermum licheniforme.* Longitudinal section of a young plant. Living material. Ehr. Haem. counterstained with wasserblau, showing the chromatin and α granules stained red and the β granules blue. *a* represents a cell almost divided; in *b*, *c*, and *d* the chromatin is still connected.

Fig. 19. *Cylindrospermum licheniforme.* Longitudinal section. Spore. K. I. Ehr. Haem.

Fig. 20. *Cylindrospermum licheniforme.* Longitudinal section of spore, showing the first division in germination. K. I. Ehr. Haem.

Fig. 21. *Cylindrospermum licheniforme.* Longitudinal section of almost mature spore and two vegetative cells. K. I. Ehr. Haem. counterstained with wasserblau, washed in acidulated alcohol. The Ehr. Haem. stains the α granules and the chromatin in the vegetative cells, but there are no α granules in the spore. The wasserblau stains the β granules.

Fig. 22. *Symploca Muscorum.* Longitudinal section. Ehr. Haem. 3 minutes, showing only chromatin in the nucleus.

Fig. 23. *Oscillatoria Okeni.* Longitudinal section. K. I. Ehr. Haem. counterstained with wasserblau. The β granules are close to the cross-walls and in the outer part of the cytoplasm.

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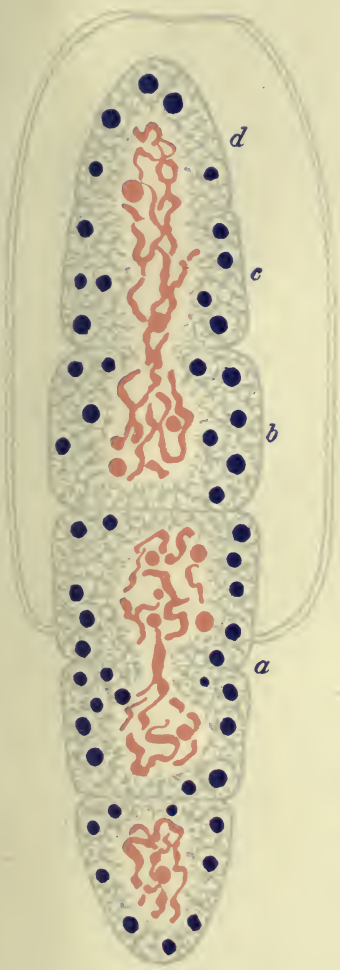

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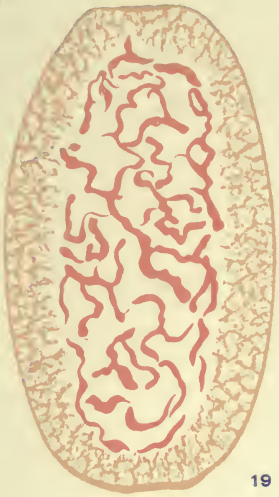

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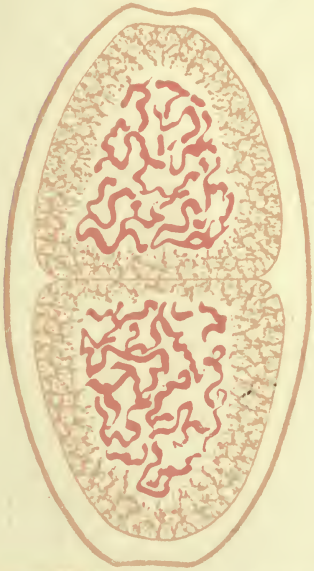

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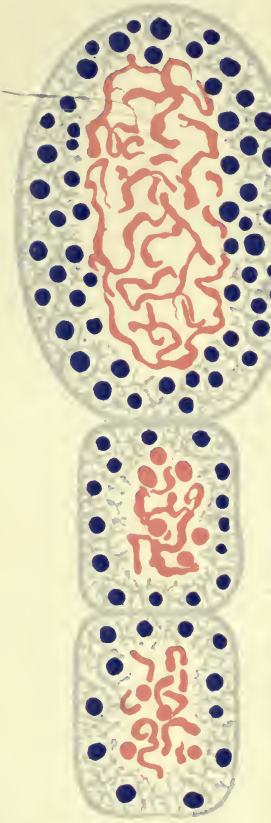

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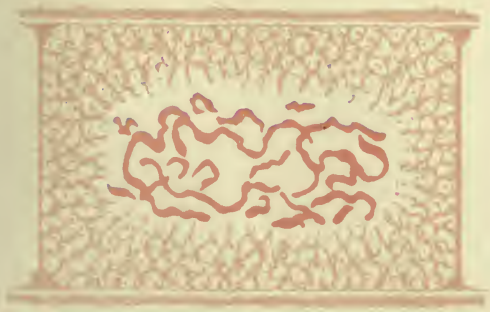

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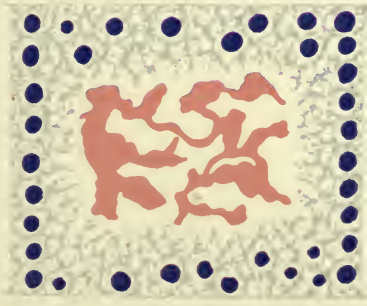

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PLATE 25.

Fig. 24. *Cylindrospermum licheniforme.* Longitudinal section of two cells and a heterocyst. Living material. Ziel's carbol fuchsin, counterstained with wasserblau. The fuchsin kills and stains the cytoplasm and the chromatin. The wasserblau restains these same parts and brings out the β granules.

Fig. 25. Same species, view and treatment as Fig. 24, except the counterstaining with wasserblau.

Fig. 26. Same species and view as Figs. 24 and 25. K. I. Aqueous methylene blue.

Fig. 27. *Oscillatoria brevis* var. Longitudinal section. K. I. Overstained with fuchsin and only partially washed out, showing the nucleus with well defined outline, but the internal structure not differentiated.

Fig. 28. *Beggiatoa arachnoidea.* Longitudinal section. K. I. washed in 95 per cent. alcohol 2 hours to remove iodine and the sulphur granules. Aqueous methylene blue, showing a continuous coarse network which extends to the surface at only a few points. The cytoplasm is made up of a very fine network around the periphery. The cell membrane is very delicate. The red granules are probably the same as the α granules in the Cyanophyceae.

Fig. 29. Cross section of same species as Fig. 28 treated in the same manner.

Fig. 30. *Oscillatoria limosa.* Cross section. K. I. Aqueous thinonin, counterstained with wasserblau and washed in acidulated alcohol. Both α and β granules are shown, and lightly stained chromatin.

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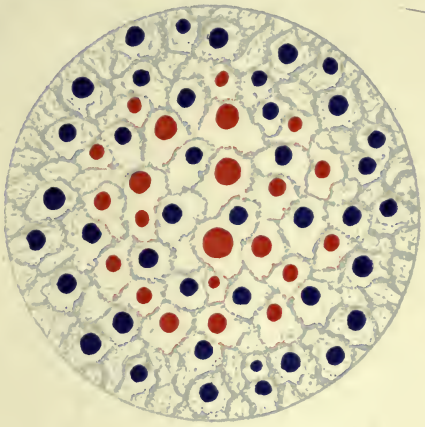

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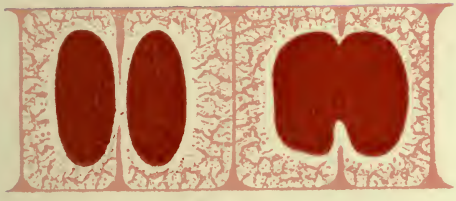

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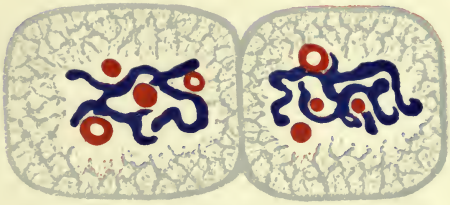

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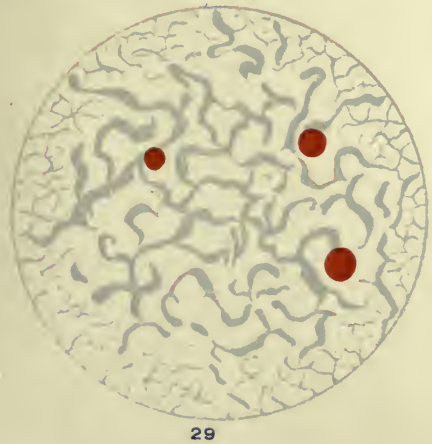

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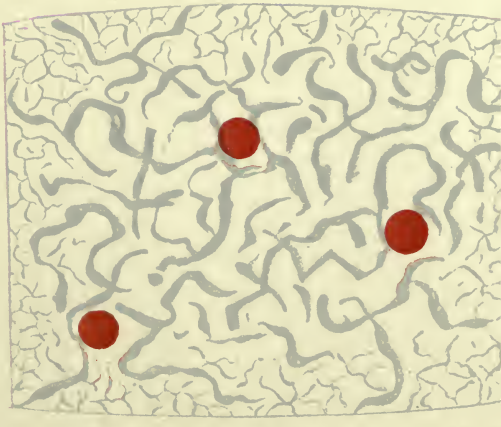

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PLATE 26.

Fig. 31. Section of a young conidium of *Dermocarpa fucicola* shortly after escaping from the parent plant. The chromatin net is beginning to form in the center of the cell.

Fig. 32. Section of a somewhat more advanced stage of *Dermocarpa fucicola* than is shown in Fig. 31. A definite chromatin network has been formed.

Fig. 33. Section of a mature plant of *Dermocarpa fucicola* in vegetative condition, showing a definite network of chromatin occupying the larger part of the cell and surrounded by a thin layer of cytoplasm.

Fig. 34. Section of *Dermocarpa fucicola* showing mature conidia.

Fig. 35. Longitudinal section of *Lyngbya aestuarii* showing the β granules along the cross-cell walls. The larger granules are along the older cell-walls, and new ones are shown arising along the new walls.

Fig. 36. *Oscillatoria chalybea.* Longitudinal view showing the α granules among the fine chromatin threads.

Fig. 37. *Cylindrospermum licheniforme.* The lower cell of the series is dividing, showing the chromatin still connecting the two daughter nuclei.

Fig. 38. *Lyngbya Lagerheimii.* Longitudinal view showing a single irregular chromatin mass and a single α granule in each cell.

Figs. 40-44. *Synechocystis aquatilis.* Showing various stages of cell division. Fig. 40, a resting cell; Fig. 41, beginning of cell division; Fig. 42, beginning of separation of the three masses of chromatin; Fig. 43, more advanced stage; and Fig. 44, two daughter cells before separation.

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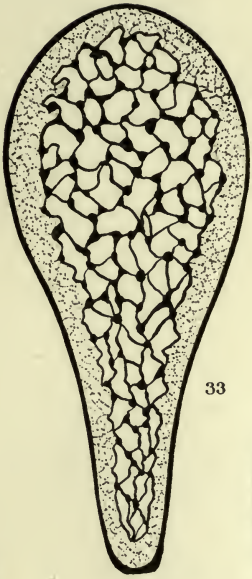
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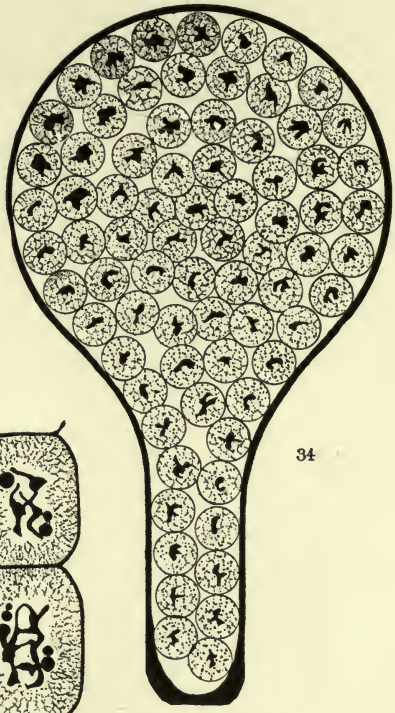
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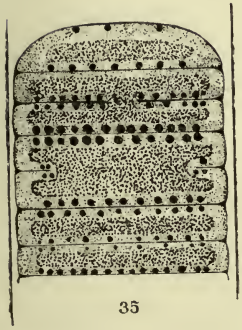
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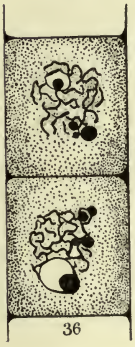
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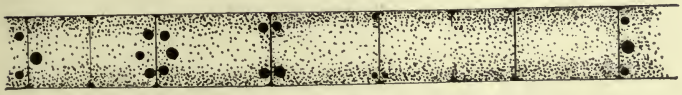
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